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Biomarkers for Environmental Monitoring in Ecotoxicology

Mary Chandana, Ch. Jhansi Lakshmi and J.Usha Rani
***Department of Microbiology, Little Flower Degree College, Uppal, Telangana State, India**

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Abstract

Ecotoxicology is a combinatorial study of toxicology in relation to ecology where in it deals with the effects of toxic substances on the health of people and other components of the ecosystem. The nature of these toxic substances can be physical, chemical or biological and these xenobiotics are increasing more than ever before. They have the potent to affect the integrity of ecosystem by disrupting the biodiversity. Therefore, early alert signs or Biomarkers provide a means for environmental monitoring by assessing the risk at an early stage. A biomarker can be defined as an alteration in biological response, starting from molecular through cellular and physiological or biochemical responses to behavioral disturbances, associated with exposure to environmental contaminants and their toxic effects. Biomarkers are mostly macromolecules like proteins, enzymes and other biomolecules like cytokines and epigenetic modifications. Apart from these, there are oxidative stress metabolites, oxidative damage to cells and antioxidants, majorly seen in plants can also be used as biomarkers in ecotoxicology. Intracellular formation of Reactive Oxygen Species (ROS) in response to environmental pollutants can result in several oxidative stress defense mechanisms. Sentinel species are the organisms which are sensitive to environmental pollutants due to their greater susceptible nature and function as ecological health indicators, providing evidences to the onset of an anthropic event, before the measurable effects are observed. Recent advancements in the field of molecular biotechnology lead to the development of modern and futuristic, highly sensitive biomarkers of exposure, effect, and susceptibility to the adverse effects of terrestrial and aquatic pollutants. Integrated usage of multiple biomarkers can greatly increase the responses of biomarkers in environmental risk evaluation.

Keywords

Ecotoxicology, Biomarkers, Proteins, Epigenetic modifications, Reactive Oxygen Species, Sentinel species, Environmental monitoring.

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INTRODUCTION:

Ecotoxicology is a newly developing science which is a combinatorial study of toxicology in relation to ecology where in it deals with the effects of toxic substances on the health of people and other components of the ecosystem. The nature of these toxic substances can be physical, chemical or biological. Increase in population, Industrial and technological development, urbanization and imprudent planning without any regard to sustainable development, have induced a variety of changes in the environment. Human activities are responsible for these changes induced in the environment in the form of pollution causing widespread damage to the living organisms in the biosphere and disruption of ecological balance.

To monitor these ecological changes, efficient and reliable monitoring systems are needed. The impact of human activities can be assessed in advance with the help of biological systems called bio-indicators/Biomarkers. Different biological systems show different levels of sensitivity.

A biomarker can be defined as an alteration in biological response of a biological system, starting from molecular through cellular and physiological or biochemical responses to behavioral disturbances, associated with exposure to environmental contaminants and their toxic effects. The process of using biomarkers in ecological risk assessment is called biomonitoring, which can be passive (sampling in-situ) or active (exposure in in-situ). The discovery and use of biomarkers is an important and developing aspect of today's epidemiological approach in improving the public health.

Range of study:

The subject of ecotoxicology includes a broad range of ecological levels of organization starting from biolmolecule to individual. Autecotoxicology and synecotoxicology deals with the population and community level issues respectively.

Biomolecule:	Understanding the biomolecular level of organization can explain the molecular mechanisms of toxicity, distinct sensitivities among individuals, and adaptation of populations to pollution. For instance, consider the biomolecular shifts in phase I and phase II reactions of organic contaminants. Contaminants such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) can be transformed by the enzymes of phase I reactions in the system of cytochrome P-450 monooxygenase. Phase I metabolites are conjugated by the enzymes of phase II which leads to detoxification. Phase I reactions complement phase II reactions. Sometimes the transformed contaminant compound may become more toxic than the parent compound.
Cells and tissues:	In presence of toxicant, the cells and tissues may undergo some changes which can be studied to effectively make use of them as biomarkers. In presence of a toxicant, the cells may modify themselves in response to the toxicant which can serve as a cellular biomarker. Cancer is a cellular response towards exposure of carcinogen. Necrosis and inflammation are some common tissue/histological biomarkers.
Organ and Organ Systems:	The effects of toxicants can also be studied at organ and organ system level of organization which are generally diverse. For instance, Low ph or high metal concentrations can lead to a change in normal ion and gas exchange of fish gills.
Whole organism:	The effect of toxicant is seen at individual level. Mortality, growth and development, behavior, physiology are commonly measured to assess the effects of toxicant at individual level.
Population and Community:	Population level impact can be studied from demographic models on the basis of vital rates such as birth, death and migration rates to estimate the consequences. Species richness or biodiversity may be used as a bioindicator for community level risk assessment.

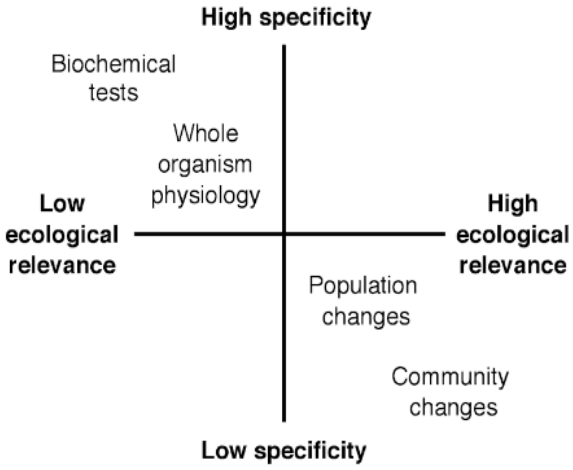

Figure 1.1 Specificity and ecological relevance of biochemical effect measurements (from the book of Principles of Ecotoxicology, third edition by C.H. Walker)

Classification of Biomarkers:

There are many classifications of biomarkers. They are broadly categorized into 3 types namely.

Biomarkers of exposure

- Internal dose
- Biologically effective dose

Biomarkers of susceptibility

Biomarkers of effect.

These three constitute a 'three-tier approach' in biomarkers usage.

Broad classification	Based on levels of organisation	Based on specificity
•Biomarkers of exposure •Biomarkers of effect •Biomarkers of susceptibility	•Biomolecular •Cellular •Physiological •Organism •Population •Community •Ecosystem	•High specificity biomarkers •Low specificity biomarkers

Biomarkers of exposure:

Biomarkers of exposure are those which indicate the exposure of an organism to the toxicant, but it does not tell us about the extent of impact that might occur due to this toxicant. Thus, these are used at hazard identification level. It involves the measuring of absorbed internal dose of the toxicant, or a xenobiotic in blood, urine, excreta, and tissue etc. through chemical analysis methods. These are used as predictors for the presence of a toxicant in a particular environment.

Some of the biomarkers of exposure are:

Type	Toxicant	Biomarker
Exposure-Internal dose	n-hexane and 2-ethoxyethanol	2,5-hexanedione and 2-ethoxyacetic acid in urine
	Xenobiotics	Parent compound or transformed compound detected in the body through proton NMR.
	polycyclic aromatic hydrocarbon (PAH) exposure	1-hydroxypyrene-glucuronide
Exposure- biologically effective dose	Alkylating agents	DNA adducts, protein adducts formation, and strand breaks (from immunoassays, 32P-postlabelling)

Validity of Exposure Biomarkers:

Validation of biomarkers is an important step because biomarkers do not always accurately predict the exposure. The analytical and toxicokinetic aspects are considered while judging the utility of a biomarker.

Biomarkers of Susceptibility:

Biomarkers of susceptibility are used to explain the susceptibility nature of an individual, or a group of individuals to the damage caused by a toxicant. Different individuals though are exposed to same environmental conditions might produce significantly different levels of biomarkers of exposure and effect. This is explained by studying the biomarkers of susceptibility. Different individuals will have different susceptibilities to the given toxicant. These differences in their susceptibilities may be attributed to genetic or non-genetic factors.

Genetic factors are again subdivided. They are: Polymorphisms that affect the expression and function of activation enzymes, Polymorphisms that affect the expression and function of detoxification enzymes, DNA repair mechanisms & others. Polymorphisms are the variations in genes among different individuals which gives rise to the phenomenon of different phenotypes in individuals who belong to the same species. As a result, the target biomolecule for a toxicant, the effective dosage and the resistance to the toxicant varies from individual to individual. This in turn is responsible for variable susceptibilities of individuals under similar conditions of environment. Non-genetic factors like age, sex, health condition, food intake, way of living, degree of exposure to the toxicant also play a key role in bringing out the differences among individuals.

Genetic based biomarkers of susceptibility are of three types mainly. They are 1. Differences in xenobiotic alteration, 2. Differences in the abilities of DNA damage repair, 3. Inherited genetic abnormalities. In case of xenobiotic alteration, the enzymes present in the body will alter the structural conformation of the chemicals that enter the body. This is called biotransformation. It might result in increased or decreased ability of the toxicant to react with the macromolecules present in the body like proteins and DNA. The enzymes which are involved in detoxification process and in activation of the toxicant differ from individual to individual. This can be explained by the following example. An organophosphate compound paraoxon is hydrolysed and deactivated by the enzyme Paraoxonase. Polymorphism in the human paraoxonase gene has two versions, one with the amino acid arginine 'A' at position 192 and the other with the amino acid glutamine at the same position. The rate of hydrolysis of Paraoxon is higher by Paraoxonase enzyme with arginine at position 192 than by the same with glutamine at position 192. This is how polymorphisms affect the metabolism of toxicants in an individual serving as potential

biomarkers of susceptibility in assessing the risk from exposure to xenobiotics.

The next type of susceptibility biomarkers is differences in the abilities of DNA damage repair. Some cells may be deficient in genes involved in DNA damage repair. It results in DNA damage through adducts formation and oncogenes activation leading to increased incidence of cancers in the individuals. The other type of susceptibility biomarker is differences in inherited genetic abnormalities. Some of the genetically acquired traits may promote the risk of cancer development thus making it easy for the toxicant to manifest its effects.

Biomarkers of Susceptibility:

Exposure to	Biomarkers
Respiratory irritants	IgA deficiency
CO, CN	Sickle cell phenotype
C6H6, Pb	Thalassemia phenotype
Cigarette smoke	Alpha-1-antitrypsin

Biomarkers of Effect or Response:

Biomarkers of effect are those which indicate the Biochemical changes in response to the exposure of a toxicant which may be seen at structural or functional level. These changes can be reversible or irreversible. Biomarkers should be efficient enough to detect and predict the upcoming anthropic event before the effects become irreversible. Some of the biomarkers of effect include altered gene expression, inhibition of enzymes and cellular alterations, immunological and behavioral responses etc. It is not easy to distinguish between biomarkers of exposure and biomarkers of effect. The biomarkers of effect are those which indicate the onset of an anthropic event, after the exposure took place and before the manifestation of disease. Following are some of the significant examples of response biomarkers.

Metallothioneins (MTs):

Metallothioneins are low molecular weight proteins (around 7000D), rich in sulfhydryl group (SH-) from cysteine and capable of binding to metals like cadmium, zinc, copper and mercury etc. In humans Metallothioneins are made of 61 amino acids and contain two metal-binding domains: alpha and beta clusters. Metallothioneins production is induced in the body by the exposure to metals. The chelate with the toxic heavy metals and protect the cells in the body from metal toxicity effects. They also protect the cells from stress-induced free radicals with their antioxidative ability. Recent studies show that metallothioneins can also be induced by non-metallic agents and physiological conditions.

Biomarkers of Oxidative damage:

Oxidative damage to the cells is caused by free radicals like reactive oxygen species (ROS) in response to oxidative stress. These are majorly seen in plants. During oxidative stress, production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) increases and damages biomolecules like DNA, proteins and lipids. Plant cells employ a wide variety of enzymatic and non-enzymatic antioxidants to counteract the production of stress radicals. Non-enzymatic antioxidants include carotenoids, tocopherols, flavonoids, Glutathione and phytochelatin etc. some of the enzymatic antioxidants include superoxide dismutases, catalase enzyme, Glutathione peroxidase (GPX), peroxiredoxins (PRX) and ascorbate peroxidase (APX). Thus, plants use different defence mechanisms to protect the cells from oxidative damage. Therefore, ROS, oxidative damage to cells and antioxidants can serve as biomarkers. Microalgal biomarkers are known for their use as xenobiotic stress biomarkers.

Biomarkers of immune system:

Xenobiotics can affect the immune system by decreasing the resistance towards infections, altering

the autoimmunity and causing hypersensitivity disorders. Biomarkers of immunotoxicity include blood count, immunoglobulin concentration in the serum, inflammatory responses and molecular biomarkers like cytokines.

Cytokines: In case of injury, infections or exposure to toxicant, an adaptive inflammatory response is triggered where soluble mediators and specialized immune cells are involved. For instance, Engineering nanomaterials (ENMs), can stimulate the inflammatory responses which vary widely. These studies can give us the pattern of cytotoxicity associated with proinflammatory responses. The Luminex Lab MAPTM system is a powerful tool for toxicity prediction and exposure assessment in proteomic biomarker profiles.

Heat Stress Proteins:

These are ATP-dependent proteins, which prevent aggregation of proteins and aid in the folding of nascent proteins and assist them to reach the sites of membrane translocation. They are called as heat shock proteins because they appear rapidly following heat stress. In presence of xenobiotics like metals, oxidizing agents, increased production of heat shock proteins can be observed. They are becoming important biomarkers in the field of ecotoxicology.

There are four types of heat shock proteins based on their molecular weight. They are

Stress 90, stress 70, chaperonins 60, hsps of low molecular weight.

Stress90 or Hsp 90 (approx. 90kDa): two forms are present, Hsp 83 and Grp 94 present in the cytosol and endoplasmic reticulum (ER) of mammals.

Stress 70 or hsp 70 (approx.70kDa): Localised in the cytosol, belongs to a multigene family and is the most widely studied hsp. Hsc 70 is constitutively expressed even in the absence of stress conditions, whereas hsp 70 isoforms expression is induced by stress. Both hsp 70 and hsc 70 help in the refolding of denatured proteins following heat shock. These are considered as ideal biomarkers.

Hsp 60 (Chaperonins 60): These are of cpn 60 family. These are organelle-specific biomarkers, localized in mitochondria and aids in protein folding.

Hsps of low molecular weight: They stabilize microfilaments by binding to actin

Western Blotting is used to study heat shock proteins best.

Exposure to	Biomarker
Organophosphates	Acetylcholine esterase inhibition
Lead	Aminolevulinate dehydratase inhibition
Xenobiotics	Heat stress proteins. hsp 90, hsp 70 etc
Transition metals	Metallothioneins and phyto chelatins induction
Oxidative stress	Antioxidative enzymes like catalase, superoxide dismutase, Glutathione etc
MCP-1	Inflammation

Epigenetics: The field of epigenetics addresses how gene expression is affected by the environmental conditions without undergoing any changes in DNA sequence. The two most important epigenetic marks widely studied are DNA methylation and histone modifications. We need them to establish patterns of gene expression in the early embryo and often persist throughout life. DNA methylation and histone modification are the most important factors in epigenetics. Addition of methyl group to the 5' 'C' of amino acid cytosine in a CpG sequence (cytosine-phosphate-guanine) is involved in DNA methylation. Histone tails can also be modified by a number of processes, like methylation, acetylation, phosphorylation. Evolutionarily conserved large family of non-coding RNAs (ncRNAs) called microRNAs

(miRNAs) are endogenous and 21- 23 nucleotides in length, bind to complementary regions of targeted transcripts and regulate gene expression by targeting mRNAs leading to mRNA degradation. They are encoded by the genome, and more than 1000 human miRNAs have been modified so far.

Ecological Risk Assessment (ERA):

Sentinel Systems: One of the most direct forms of monitoring is to exploit biological systems already present in the environment, called sentinel biomonitors.

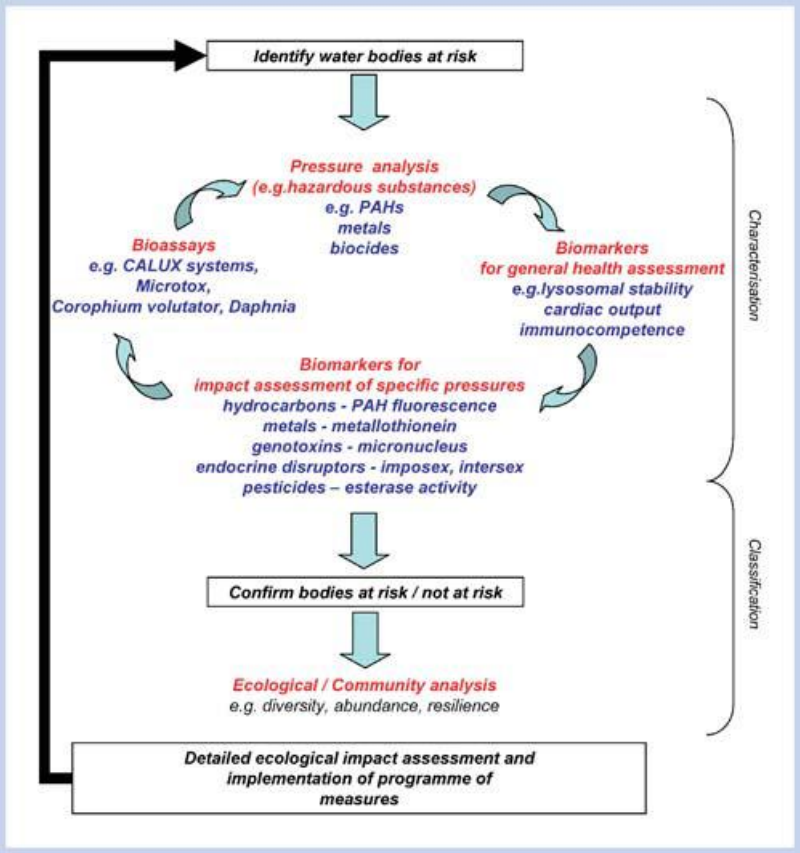

Fig 1.2. Description of hierarchal approach to risk assessment. Adapted from Stagg and McIntosh (1998), Environment Agency (2002), Galloway et al. (2004a), and Lehtonen (2005b).

CONCLUSION:

The development of Biomarkers in ecotoxicological studies is a growing need. These can be successfully employed as ecological indicators (bioindicators) to assess and predict environmental change in a timely manner. One biomarker alone may not be sufficient to monitor the environmental quality. Usage of multiple biomarkers through multivariate and integrative approach will increase the specificity and accuracy of the results obtained. In general, biomarkers show a number of dose response relationships that are the result of all possible interactions of all the contaminants present in the ecosystem under study. The health condition of the population being studied can be assessed by the multiple response concept. More is the concentration of the toxicant and exposure time; more are the responses at higher levels. Every response, at every level of complexity (biomolecular, cellular, physiological, organism level etc.) is considered a biomarker. Therefore, the use of

biomarkers allows us to identify the level of risk to the populations.

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